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Sendai virus induces high levels of tumor necrosis factor mRNA in human peripheral blood leukocytes

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ABSTRACT

Sendai virus induces human peripheral blood leukocytes to produce high levels of tumor necrosis factor (TNF) mRNA. TNF mRNA can represent as much as 0.6% of the total mRNA. Kinetic studies indicate that the level of TNF mRNA peaks about 2 hours before that of IFN-α mRNA produced in the same system. Although the peak levels of TNF and IFN-α mRNA were similar, TNF in the culture supernatants was at a 200 fold lower level than IFN-α. Cloning and sequence analysis of TNF cDNA isolated from peripheral blood leukocytes RNA showed that normal human cells in response to Sendai virus produce TNF identical to that previously isolated and cloned from tumor-derived cell lines. A bacterial expression system was used to produce the cloned TNF at a maximum level of 2 X 10^6 units per ml of culture.

INTRODUCTION

Tumor necrosis factor (TNF) is a protein secreted by macrophage/monocyte cells in response to invasive stimuli. It was so named for its ability to elicit hemorrhagic necrosis of tumors in injected mice (1). Because of its potential as an antineoplastic agent, considerable attention has been focused on this protein. Recently, human TNF has been purified to homogeneity (2), cloned, and expressed in *E. coli* (3-8).

The availability of recombinant TNF in large quantities has permitted a detailed examination of the properties of this protein. In addition to the historically studied cytotoxicity toward neoplastic tissue, a broad spectrum of activity has now been attributed to this molecule. TNF can participate in the inflammatory response by altering host metabolism and thereby mediating cachexia (9), activating polymorphonuclear neutrophil phagocytic and cytotoxic functions (10), enhancing eosinophil toxicity (11), and modulating the procoagulant properties of

vascular endothelial cells (12, 13). TNF can also stimulate bone resorption (14) and fibroblast growth (15).

TNF is rapidly produced in response to bacterially-derived endotoxins (1), suggesting that TNF may play an initial role in the host's defense against bacterial infection. Although TNF production in response to other infectious agents, such as animal viruses, had not been reported, we discovered during the study of cytokines induced in human peripheral blood leukocytes (PBLs) by Sendai virus that these cells were producing what appeared to be surprisingly high levels of TNF mRNA. Despite this, cytotoxic activity in the culture medium was at a low level. Since the previously cloned TNF was generated from endotoxin and phorbol myristate acetate induced tumor-derived cell lines, and since multiple cytotoxic TNF-like proteins may exist (4, 16-18), it was necessary to determine whether Sendai virus, a previously unknown inducer of TNF, was directing normal human cells to express TNF and whether the amino acid sequence of the expressed protein was identical to that of the previously cloned TNF. We compare the temporal expression of TNF mRNA and secreted cytotoxic activity to interferon-α (IFN-α) mRNA and IFN-α proteins in Sendai virus-induced PBLs. We report the levels of TNF and IFN-α mRNAs and calculate their frequencies in induced PBL mRNA.

MATERIALS AND METHODS

Materials

Polymyxin B, chromatographically purified lipopolysaccharide (LPS) from *E. coli* 0111:B4, and sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis molecular weight standards were obtained from Sigma Chemical Co. Pure natural IFN-α (specific activity 2.7 X 10^8 units/mg) was purchased from Interferon Sciences. The P_L promoter and a gene for a temperature sensitive repressor from the bacteriophage λ were obtained from Dr. Steven J. Sandler and were ligated with a consensus ribosomal binding site and pUC9 to form an expression vector pUC9-PL85 by L. Cheryl Hendrix and Dr. Rajinder Sidhu. SP6 RNA polymerase and transcription vectors pGEM-1 and pGEM-2 were from Promega Biotec. Mouse anti-TNF serum made against

human recombinant TNF was supplied by Dr. Rita Chen. Endotoxin assays were performed with the Limulus Amebocyte Lysate endotoxin assay kit from M.A. Bioproducts.

Oligonucleotides were synthesized using phosphoramidite chemistry with a Systec Microsyn 1450 synthesizer. They were purified as described (19). Oligonucleotide A (dATTGATCTCA-GCGCTGA) is complementary to TNF mRNA. It was ^{32}P-labeled as described (19). Oligonucleotides B (dGATCTATGGTTCGTTCTTCTTC-CCGTACC) and C (dTCGGGGTACGGGAAGAAGAACGAACCATA) share partial complementarity and encode a start codon and the first 8 amino acids of mature TNF. Codons were selected on the basis of *E. coli* codon usage preference and secondary structure considerations.

Preparation and Induction of PBLs

Pooled PBLs from 20-170 healthy human donors were prepared by treatment with ammonium chloride and cultured as described (20). Cultures containing 10^7 cells/ml were primed at -2 hours with 100 units/ml of either crude IFN-α (20) or pure IFN-α as indicated. At 0 hour Sendai virus was added to a concentration of 200 hemagglutination units/ml and incubation of the cultures was continued at 37°C. At the times indicated cultures were centrifuged, and cells were immediately processed for RNA isolation. Culture supernatants were stored at -70°C.

IFN-α and Cytotoxicity Assays

IFN-α was measured (duplicates agreed within 10%) with a radioimmunoassay (BRL) using the monoclonal antibody YOK-5/19. Activity is expressed in international units. Cytotoxic units were measured at least in triplicate (standard deviations were usually 15% and not more than 28% using L929 cells (21) after heating culture supernatants at 60°C for 30 minutes. One unit is the amount required to give 50% cytotoxicity in the standard assay. A laboratory reference recombinant TNF standard was included with all assays.

Preparation and Screening of cDNA Library

RNA was extracted from PBLs cultured for 4 hours after virus addition using a guanidine isothiocyanate solution (22), purified over cesium chloride gradients, and fractionated with an oligo(dT) cellulose column to give poly(A)$^+$ RNA. A cDNA

library was prepared (23) on nitrocellulose filters (24) using the PstI site of pBR322 and X1776 cells. Duplicate replica library filters were prepared and hybridized with ^{32}P-oligonucleotide A or a nick-translated cDNA fragment from clone pBR322-TNF1 as described (19). Clone pBR322-TNF1 contains the cDNA sequence for TNF from base numbers 371 to the 3' end of the mRNA (3) and is described in the Results section.

Sequencing and Expression in E. coli of TNF cDNA

Nucleotide sequencing of the cDNA clone pBR322-TNF1 was done by a combination of methods (25, 26). Both strands of the entire mature structural gene were completely sequenced.

The AvaI-EcoRI fragment encoding most of the mature TNF gene from clone pBR322-TNF1 was linked to the large BglII-EcoRI fragment of pUC9-PL85, derived after EcoRI and partial BglII digestion, by the two complementary oligonucleotides B and C. E. coli JM83 cells with and without the resulting TNF expression vector, pUC9-PL1-TNF1, were grown in 2xYT medium at 30°C and at OD_{600} = 1.2 were either maintained at 30°C or shifted to 42°C for an additional 2 hours. Cells were lysed by treatment with lysozyme and freeze-thawing, and soluble extracts were applied to 15% SDS-polyacrylamide gels (27). Gels were stained with the Kodavue Electrophoresis Visualization Kit (Kodak).

RNA Dot Blots

Dot blots of total RNA isolated as described above were made (19) and hybridized under stringent conditions (28) to ^{32}P-RNA probes synthesized with SP6 RNA polymerase after insertion of the indicated cDNA insert fragments into transcription vectors pGEM-1 and pGEM-2. The filters were washed in 2X SSC at room temperature for 10 minutes with rocking. This wash was repeated twice. The filters were then incubated (30 minutes, room temperature with rocking) in the same solution plus 1 µg/ml of RNase A, washed once in 0.1X SSC containing 0.1% SDS at 74°C for 30 minutes, dried and exposed to X-ray film. Standard curves of signal intensity versus RNA content were generated using RNA dots made from TNF or IFN-α mRNA synthesized by SP6 RNA polymerase after insertion of the entire TNF cDNA insert from clone pBR322-TNF1 into pGEM-1 or an 1100 base pair (bp) cDNA insert for IFN-αD into pGEM-2. All

dots were quantitated by densitometry with a Beckman DU-8 gel scanning system.

Northern Hybridization Analysis

Total RNAs were electrophoresed through 1.5% agarose/formaldehyde gels and transferred to a nitrocellulose filter as described (29). The blot was hybridized under stringent conditions (28) to a ^{32}P-RNA probe synthesized with SP6 RNA polymerase using pGEM-1 containing a cDNA fragment coding for bases 371 to 1171 (3) from clone pBR322-TNF1 as the transcription vector. The filter was hybridized and washed as described for the RNA dot blots except that the filter was not treated with RNase A.

RESULTS

Isolation of TNF cDNA Clones

An oligonucleotide probe complementary to the mRNA for the TNF cloned from the promyelocytic leukemia cell line HL-60 (Probe A) was used to test for TNF expression in cultured human PBLs after induction by Sendai virus. The strong hybridization signal (not shown) seen with induced PBL poly(A)$^+$ RNA, isolated 4 hours after virus infection, indicated that TNF mRNA was induced to what appeared to be a high level. However, a cytotoxicity assay of PBL culture supernatants taken 20 hours after virus infection showed that the total possible cytotoxic activity due to TNF was not more than 300 units/ml. The presence of virus in the culture supernatants gave erroneously high activity in the cytotoxic assay (Table 1). This effect persisted even for samples collected 20 hours after infection. Viral toxicity was eliminated by heating the samples at 60°C for 30 minutes, a treatment which did not affect TNF activity (Table 1).

To determine if the hybridization signal with the TNF oligonucleotide probe was due to the RNA for TNF or a TNF-like protein, a cDNA library was made with RNA isolated from virally-induced PBLs and screened with Probe A. The identified positive clones were confirmed to be TNF-related after hybridization with two additional TNF oligonucleotide probes. All 5 positive clones appeared to be closely-related to the

Table 1. Presence and elimination of L929 cell cytotoxicity due to virus in PBL culture supernatants.

Sample	Heat treatment[a]	Cytotoxic activity (units/ml)
Culture medium	+	0
Recombinant TNF	-	128 ± 16
Recombinant TNF	+	131 ± 19
0 hour supernatant before virus addition	-	12.7 ± 5.8
0 hour supernatant before virus addition	+	6.3 ± 5.7
0 hour supernatant after virus addition	-	589 ± 165
0 hour supernatant after virus addition	+	6.9 ± 5.3
20 hour supernatant	-	686 ± 47
20 hour supernatant	+	229 ± 42

[a]Samples were heated for 30 minutes at 60°C

HL-60 TNF, since restriction enzyme cleavage generated fragments of the expected length. Nucleotide sequencing of the clone with the largest cDNA insert, pBR322-TNF1, which extends from base number 371 to the 3' end of the TNF mRNA (3), showed that the mRNA sequence for mature TNF produced by normal human PBLs induced with Sendai virus was identical to that produced in the HL-60 (3, 4) and U-937 (6) cell lines after induction with either unspecified agents or with phorbol esters and endotoxin, and probably transcribed from the isolated genomic gene (5).

Expression of TNF cDNA in E. coli

The TNF cDNA was engineered for expression in E. coli using a vector containing the P_L promoter, a gene for a heat sensitive repressor, and a consensus ribosomal binding site. Only after a shift in temperature to 42°C in E. coli containing the expression vector pUC9-PL1-TNF1 could a 17,300 dalton protein be seen after SDS-polyacrylamide gel electrophoresis of proteins extracted from the E. coli (Fig. 1). We have obtained up to 2 X

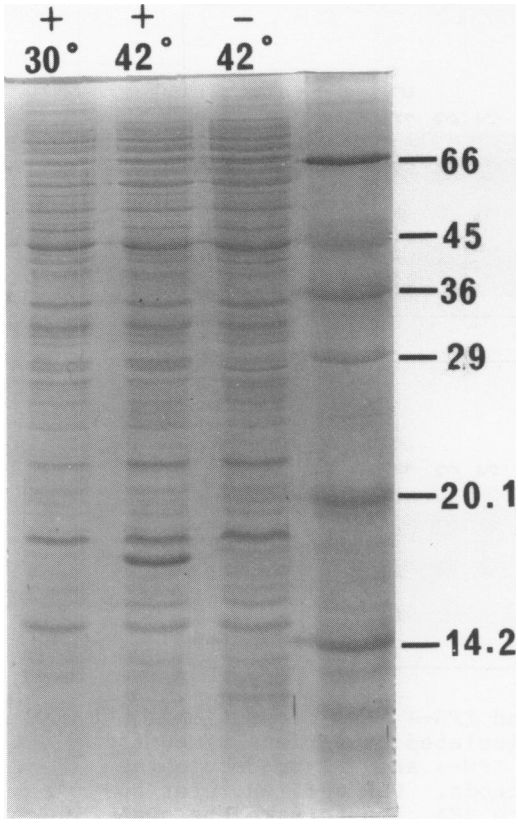

Fig. 1. SDS-polyacrylamide gel electrophoresis of E. coli extracts. E. coli JM83 with (+) or without (-) the TNF expression vector were grown at 30°C and at OD_{600} = 1.2 were either maintained at 30°C or shifted to 42°C for an additional 2 hours. Cell extracts were applied to SDS-polyacrylamide gels. The right lane contains molecular weight standards (X 10^{-3}).

10^6 units/ml of cytotoxic activity when cultures were grown for 3 hours after the shift to 42°C at OD_{600} = 2.5. The recombinant human TNF was purified to a specific activity of 1 X 10^7 units/mg and used to generate TNF antiserum and in the TNF half-life study described below.

Frequency and Kinetics of Induction of TNF and IFN-α mRNAs

Although extracellular cytotoxic activity was at a low level in the PBL cultures, clearly TNF mRNA was transcribed. With the availability of the TNF cDNA we could confirm that TNF mRNA was at a high level in the virus-induced PBLs. First, we screened duplicate filters of the cDNA library (20,000 colonies) with the cDNA probe. Duplicate positives were found for 0.05% of the colonies. Second, TNF mRNA frequency in the PBLs was directly quantitated by dot blot hybridization of the 4 hour

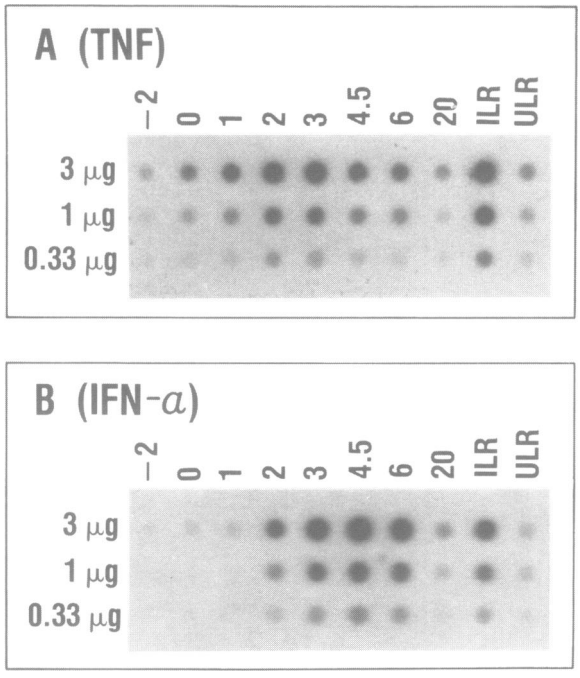

Fig. 2. Time course of TNF and IFN-α mRNA production. Dot blots were made of total RNA isolated at various times from PBLs prepared and induced for IFN-α and TNF production as described in Materials and Methods. ULR and ILR refer to uninduced and induced leukocyte RNA used to make the cDNA library. Numbers above the dots are hours after addition of virus. RNA was quantitated by comparison to dots made with the appropriate standard RNA (not shown, see Materials and Methods). (A) ^{32}P-RNA probe for TNF mRNA was made after insertion of a cDNA fragment coding for bases 1171 to 1643 (3) from clone pBR322-TNF1 into pGEM-2. (B) ^{32}P-RNA probe for IFN-α mRNA was made after insertion of a 1100 bp cDNA insert for IFN-αD into pGEM-2.

induced total RNA (ILR, Fig. 2A) with a TNF RNA probe made with SP6 RNA polymerase. Standard curves of TNF mRNA content versus signal intensity were generated using dots made with TNF mRNA synthesized in vitro by SP6 RNA polymerase. The frequency of TNF mRNA was 0.11%, assuming that mRNA represents 2% of the total PBL RNA. Thus, the two methods of RNA frequency measurement gave similar results.

In the experiment above the TNF mRNA content of the PBLs was analyzed at a time (4 hours after virus infection) chosen to

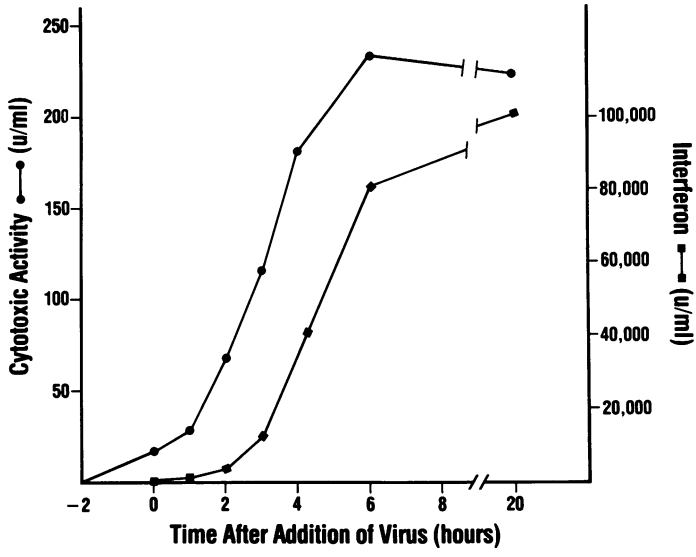

Fig. 3. Time course of TNF and IFN-α production. Cell supernatants were isolated at various times from virus-induced PBL cultures and assayed for cytotoxic activity and IFN-α as described in Materials and Methods.

optimize IFN-α mRNA levels. Since the kinetics of IFN-α and TNF production may be distinct, we looked at the levels of the respective mRNAs (Figs. 2A and 2B) and activities (Fig. 3) at various times for both IFN-α and TNF in primed virus-induced PBLs. Dot blot hybridization of total RNA samples with RNA probes showed that a small increase in TNF mRNA had already occurred before the addition of virus at 0 hour. Possibly a low level spontaneous induction of TNF had occurred. Unlike mouse cells, human monocytes can produce a small amount of TNF in the absence of known inducers (30). It is also possible that endotoxin, an established TNF inducer, in the culture medium could be responsible for the early low level induction of TNF. The maximum level of TNF mRNA was reached 2 hours after virus infection, approximately 2 hours before the peak level of IFN-α mRNA was obtained. For the induction shown, frequencies of the mRNAs at the peak levels were similar, 0.15% for TNF and 0.13% for IFN-α. As described above, signals were quantitated against standard dots made with either TNF or IFN-α mRNA synthesized

Table 2. Absence of TNF inhibitor in PBL culture supernatants

Sample	Cytotoxic activity (units/ml)
Recombinant TNF	131 ± 19
0 hour supernatant after virus addition	17 ± 2.6
0 hour supernatant after virus addition plus recombinant TNF	160 ± 44
4.5 hour supernatant	180 ± 29
4.5 hour supernatant plus recombinant TNF	360 ± 65
20 hour supernatant	229 ± 42
20 hour supernatant plus recombinant TNF	354 ± 32

in vitro with SP6 RNA polymerase. In certain experiments where 2 fold higher levels of cytotoxic activity were produced, 4 fold higher TNF mRNA levels were seen. As negative controls, dots of yeast 18S and 28S ribosomal RNA and rat liver total RNA were also hybridized. No signals were seen with these samples (data not shown).

IFN-α activity was produced as expected (Fig. 3). No cytotoxic activity was present in the cultures at -2 hours, but a low level (20 units/ml) was detectable before virus addition at 0 hour. The cytotoxic activity reached a half-maximal level approximately 90 to 120 minutes before IFN-α did. Despite the similar specific activities of TNF (2) and IFN-α (31) the maximum level of cytotoxic activity was more than 200 fold lower than that of IFN-α.

Characterization of Cytotoxic Activity

The cytotoxic activity present at 0, 6, and 20 hours in the supernatants from IFN-α primed virus-induced PBL cultures was completely neutralized by treatment for 4 hours at 4°C with mouse anti-TNF serum made against purified recombinant TNF. These results indicate that most, if not all, of the cytotoxic activity was due to TNF.

Since the mRNAs for IFN-α and TNF were at similar levels, but the apparent level of TNF production was much lower than that of IFN-α, we tested for the presence of an inhibitor in the culture supernatants which might interfere with the cytotoxicity assay. A mixing experiment with recombinant TNF (Table 2) excluded the presence of an inhibitor in the culture supernatants.

Since only steady-state levels of TNF were measured in the culture supernatants, it is possible that the difference in apparent production levels of TNF and IFN-α is actually due to a much shorter half-life of secreted TNF than IFN-α. A rapid TNF destruction or uptake by blood cells would have implications in the clinical use of TNF for the treatment of cancer patients. To test this possibility, recombinant TNF was added to a concentration of 800 or 8000 units/ml to IFN-α primed PBL cultures just after the addition of virus at 0 hour. The levels of TNF in culture supernatants were measured over a 20 hour period. The expected increase in activity was observed in the culture spiked with 800 units/ml of TNF, and at no time in either culture was a significant loss of activity (more than 20%) observed (data not shown). Thus, the half-life of secreted TNF in PBL cultures is greater than 20 hours, provided that recombinant TNF has identical stability and receptor binding properties as natural TNF.

Effect of Various Induction Conditions on TNF mRNA and Activity

To confirm that Sendai virus was the inducing agent for TNF in the described experiments, PBL cultures were treated in various ways, and cytotoxic activity and TNF mRNA levels were monitored (Fig. 4). In cultures which were not primed or treated with virus, TNF was produced at a low level (as evidenced by a modest increase in both TNF mRNA and cytotoxic activity). The uninduced level of cytotoxic activity varied considerably from experiment to experiment with peak levels (20 to 140 units/ml) most often occurring at 2 hours. The activity in these cultures usually completely disappeared by 20 hours. The greatest induction of TNF occurred in the virus-treated cells (peak levels of 200 to 500 units/ml), and priming of the

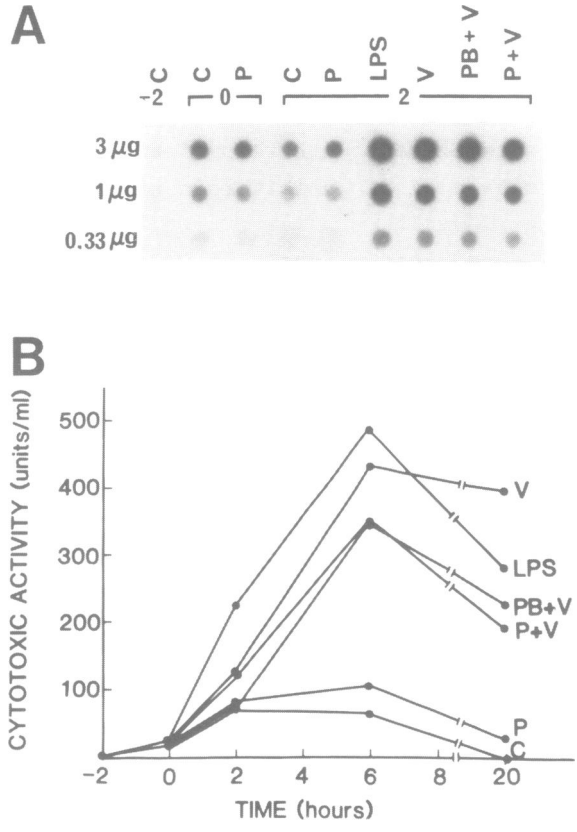

Fig. 4. Effect of induction conditions on production of TNF mRNA and activity. PBLs were prepared and cultured as described in Materials and Methods. Before the addition of cells some culture media was treated for 30 minutes with 25 μg/ml polymyxin B (PB). At -2 hours some cultures were primed (P) with 100 units/ml of pure natural IFN-α, and at 0 hour cultures were either not treated (C) or induced with 200 hemagglutination units/ml of Sendai virus (V) or with 10 μg/ml lipopolysaccharide (LPS). (A) Dot blots of total RNA isolated as indicated at -2, 0, or 2 hours after addition of virus to induced cultures were hybridized with a ^{32}P-RNA probe for TNF made using SP6 RNA polymerase after insertion of a cDNA fragment coding for bases 371 to 1171 (3) from clone pBR322-TNF1 into pGEM-1. RNA was quantitated by comparison to dots made with standard TNF RNA (not shown, see Materials and Methods). (B) Cytotoxicity of culture supernatants isolated at various times was measured as described in Materials and Methods.

cells with pure IFN-α had no effect on TNF production. Generally, a decrease (30 to 40%) in virus-induced TNF levels occurred between 6 and 20 hours after infection. Over six experiments, the peak levels of TNF mRNA and cytotoxic activity observed in virus-infected PBLs were 2 to 6 fold and 2 to 20 fold higher, respectively, than in uninfected cells. Polymyxin B did not block either the background production of TNF in the uninduced cultures (data not shown) or the virus-induced increased levels of both TNF mRNA and activity (Fig. 4), indicating that low levels of endotoxin (2 ng/ml) in the culture medium were not responsible for the background TNF induction. This antibiotic has been shown to prevent induction of TNF by endotoxin (32). Endotoxin (LPS) at 10 μg/ml induced TNF mRNA and cytotoxic activity in PBLs to levels similar to those seen after virus induction.

An antibody neutralization test was done on the 6 hour culture supernatants described in Fig. 4. The cytotoxic activity present in all samples was completely lost after treatment for 4 hours at 4°C with mouse anti-TNF serum made against recombinant TNF. Thus, Sendai virus, endotoxin, and background induced cytotoxic activity in PBLs is due to TNF. Control mouse serum had no effect on the cytotoxic activity in any of the samples.

The experiments described above show that TNF is produced by PBLs in response to Sendai virus. However, the level of TNF is relatively low (approximately 200 fold lower) in comparison to the level of IFN-α induced by the same agent, even though the mRNA levels for the two proteins are similar. Northern hybridization analysis was performed (Fig. 5) to further characterize the hybridization signal observed for the samples described in Fig. 4. The hybridization signal is apparently due to one mRNA species with a size of 18S. This size is consistent with that of the TNF mRNA observed in HL-60 cells induced with phorbol esters (3). In PBLs the same mRNA species is apparently induced by background conditions, endotoxin, or by Sendai virus. The relative intensities of the signals for the various samples were as expected from the dot blots (Fig. 4).

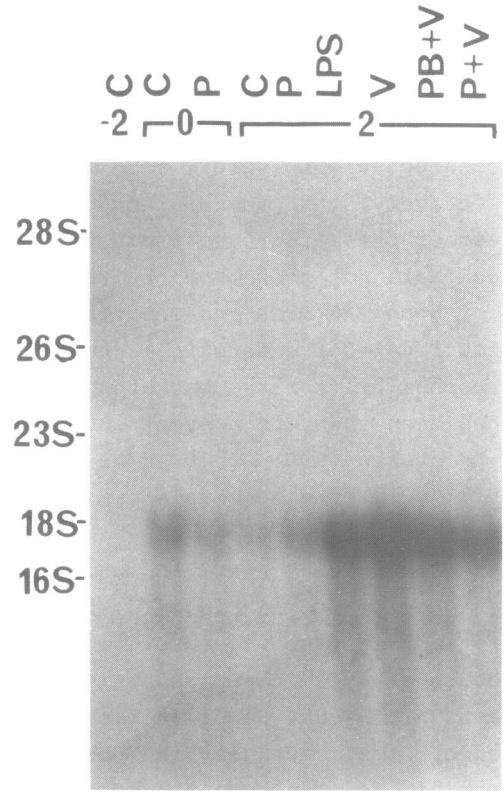

Fig. 5. Northern hybridization analysis of TNF mRNA produced under various induction conditions. PBL cultures were treated as described in Fig. 4. The Northern was made and hybridized to a ^{32}P-TNF RNA probe as described in Materials and Methods. The positions of RNA standards are shown at the left.

DISCUSSION

Cytotoxins produced by the immune system are a heterogeneous group of proteins, the nature of which depends upon the variety of inducing agent and target cell involved (16-18, 33). As the cytotoxin TNF was originally named for a protein produced in endotoxin-treated mice (1), most studies of TNF have focused on induction by endotoxin, although certain mitogens and phorbol myristate acetate have also been shown to induce TNF (34). Even though multiple closely-related TNFs may exist (4, 17), the deduced amino acid sequences for mature TNF

cloned from the tumor-derived HL-60 (3, 4) and U-937 (6) cell lines and lung macrophages (7) induced with phorbol myristate acetate and endotoxin were identical. We have shown in this study that normal human PBLs also express TNF of the same amino acid sequence in response to an inducer of a different nature. We have found that Sendai virus not only induces IFN-α as expected, but also induces TNF. Since IFN-α has previously been shown not to be an inducer of TNF (34), it is unlikely that TNF is produced in our system in response to the induced IFN-α. In addition, we have shown that priming PBL cultures with 100 units/ml of IFN-α does not give an increase in TNF expression as compared to unprimed cultures (Figs. 4 and 5). This co-induction of TNF and IFN-α may be physiologically significant, since IFN-α can increase the number of TNF receptors on cell surfaces (35). However, the mechanism of induction for each protein must contain unique elements, since all inducers of IFN-α do not induce TNF (34) and we found that IFN-α was not expressed in cultured PBLs in response to endotoxin (data not shown).

A recent study (36) has also shown that Sendai virus induces peripheral blood mononuclear cells to express a cytotoxin, which is believed to be TNF on the basis of neutralization of cytotoxic activity with a recombinant TNF monoclonal antibody. That report, however, may offer evidence for the existence of a cytotoxin closely-related to the cloned TNF, since the majority of the cytotoxic activity in that study eluted on a gel filtration column with a relative molecular mass of approximately 14,000 daltons. Cloned TNF has a monomer size of 17,000 daltons and usually behaves as a 40,000 to 45,000 dalton multimer on gel filtration columns (2, 5, 33).

We have found that after induction by Sendai virus, PBLs produce a high level of TNF mRNA. The frequency of TNF mRNA can be as high as 0.6%. Since PBLs are a mixed population of cells, presumably not all of which are producing TNF, this frequency is very high in comparison with the estimated frequency of TNF mRNA for HL-60 cells of 0.0035% (3) and 0.002% (4) and for U-937 cells of 0.01% (6), where other inducing agents (including phorbol esters and endotoxin) were used.

We were surprised, therefore, to find that although TNF mRNA was abundant, TNF was present only at low levels in PBL culture supernatants. For a direct comparison, we looked at IFN-α mRNA and protein levels, since IFN-α is also induced in the same system by Sendai virus. Although the levels of mRNAs for the two proteins were similar, there was about 200 fold less TNF than IFN-α in the culture supernatants. The apparent discrepancy between mRNA content and secreted TNF level is not due to a TNF inhibitor present in the culture supernatants nor to a rapid destruction or uptake of secreted TNF. Perhaps inefficient TNF mRNA translation or post-translational modification is responsible for the lower production of TNF relative to IFN-α. The unusually long leader sequence (76 amino acids) of TNF might be less efficient in intracellular routing.

A post-transcriptional control mechanism may be regulating TNF levels. In a study on mouse cachectin (TNF), peritoneal macrophages appeared to contain a pool of untranslated TNF mRNA (37), although a kinetic analysis was not reported. The addition of endotoxin to these cells increased TNF transcription three fold and initiated TNF translation. If a controlled translational block exists in our system, it is not released by endotoxin, since LPS-treated PBLs also produce low levels of TNF when TNF mRNA levels are high. Perhaps a cell-cell interaction signal is required to increase TNF translation and secretion. This mode of control would both keep the general concentration of TNF low and deliver high concentrations upon presentation of the target cell.

What are the physiological roles of TNF? TNF, like IFN, increases the expression of HLA-A, B antigens (38) suggesting that TNF may enhance the participation of cytolytic T lymphocytes in the destruction of neoplastic or virally-altered cells. In addition, TNF *in vitro* stimulates an increase in the phagocytic abilities of polymorphonuclear cells (10), cells which are capable of virus particle inactivation and tumor cell destruction (39, 40). Recently, TNF was shown to protect "aged" human fibroblasts from the cytopathic effect of encephalomyocarditis virus (41). The antiviral state was

mediated by the induction of IFN-β_2. A role for TNF in the host's defense against infectious agents is now a more intriguing possibility since, as shown here, virus increases TNF expression.

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